Fisheries and Oceans Canada | Pêches et Océans Canada
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National Capital Region
Canadian Science Advisory Secretariat
Science Advisory Report 2012/070

GUIDELINES ON DEFINING POTENTIAL EXPOSURE AND ASSOCIATED BIOLOGICAL EFFECTS FROM AQUACULTURE PEST AND PATHOGEN TREATMENTS: ANTI-SEA LICE BATH TREATMENTS IN THE BAY OF FUNDY, NEW BRUNSWICK

Green fluorescein dye, used to measure therapeutant transport and dispersal, being released from an Atlantic salmon net pen following an anti-sea lice tarp bath treatment. (courtesy of Fred Page, DFO, St. Andrews Biological Station)

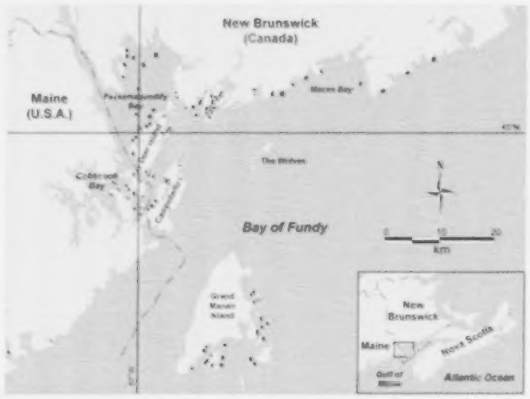

Figure 1. Map of southwestern New Brunswick, showing approved finfish farms in 2010.

Context:

Cultured Atlantic salmon are susceptible to infectious bacterial and viral diseases and to infestations by parasites, such as sea lice. Sea lice are ecto-parasites which can pose a problem for the salmon aquaculture industry, and while minor infestations are not harmful to fish, as sea lice levels increase, so does the potential damage to the fish. If left untreated, heavy sea lice loads can affect the fish's physiology and behaviour, and increase the risk of death due to secondary infections. As such, salmon aquaculture operators require means for controlling sea lice abundance within net pens.

Therapeutants are one important tool for controlling sea lice on farmed salmon. Therapeutants used in the aquaculture industry are considered either drugs or pesticides depending on the application method. Products applied topically or directly into water are considered pesticides, while, products delivered through medicated feed or by injection, are considered drugs. Pesticides are regulated by Health Canada's Pest Management Regulatory Agency (PMRA) under the Pest Control Products Act, and drugs are regulated under the Food and Drugs Act which is administered by Health Canada's Veterinary Drug Directorate.

This national advisory process involved a peer review of three research working papers aimed at defining the potential exposure and biological effects on non-target species from sea lice bath treatment pesticides currently, or recently, used in the aquaculture industry in the Bay of Fundy, New Brunswick. These were: Salmosan® 50WP (active ingredient (a.i.): azamethiphos), Interox-Paramove® 50 (a.i.: hydrogen peroxide) and AlphaMax® (a.i.: deltamethrin). This process was undertaken to assess the research and analysis performed to date in order to provide scientific advice to regulators and policy makers within Fisheries and Oceans Canada and the Pest Management Regulatory Agency.

This Science Advisory Report is from the Fisheries and Oceans Canada, Canadian Science Advisory Secretariat, national advisory meeting of November 2–3, 2011 in St. Andrews, NB on Defining Potential

Canada

Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments. Additional publications from this process will be posted as they become available on the DFO Science Advisory Schedule at http://www.dfo-mpo.gc.ca/csas-sccs/index-eng.htm.

SUMMARY

An internal peer review meeting was held to assess preliminary results from research related to three anti-sea lice pesticides ("bath treatments") currently, or recently, used by the salmon aquaculture industry in the Bay of Fundy, New Brunswick in order to provide scientific advice to regulators and policy makers. This is the first meeting in a two-part process looking at the potential for key non-target organisms to be exposed to bath treatment effluents and for that exposure to result in biological effects. Part two of this process, anticipated for 2013, will be broader in scope and include a full CSAS Science Advisory Process. Specifically, this review examined: 1) field data and initial models of potential pesticide exposure following bath treatments; 2) the toxicity of three pesticides on three non-target species indigenous to the Bay of Fundy, New Brunswick, and their various life stages and; 3) preliminary estimates of the potential biological effects based upon the predicted exposure profile.

The following is a summary of the advice and conclusions reached during the peer review meeting:

- Numerous physical, chemical, operational and husbandry factors were identified that can influence the exposure profile following pesticide bath treatments.
- Based on dye dispersion studies, the therapeutant plume shape following release of treated water from tarp and well boat bath treatments is generally elliptical. The area enclosed within the ellipses associated with tarp releases increases with time in a manner that is consistent with the diffusion diagrams of Okubo (1971, 1974).
- Concentrations of therapeutants that are greater than levels of laboratory derived biological effects (i.e., LC_{50} and NOEC) can extend tens to thousands of meters (i.e., a few kilometres) away from the effluent source over a 1–3 hour period. These distances depend upon the circulation, toxicity of the individual therapeutant and the quantity of therapeutant used.
- Well boat discharge configurations vary with vessel. Following release from vessels with horizontally directed discharges, the plume initially follows a typical jet dynamic which evolves into the standard turbulent transport and dispersal regime.
- Laboratory studies show that acute lethal toxicity (LC_{50} after 1 hour exposure) levels vary with the therapeutant, exposed species and life stage.
- American lobster, a commercially important species, was consistently more sensitive to therapeutants than Crangon and Mysid shrimps tested.
- There is evidence of sub-lethal and delayed effects in adult American lobsters from Salmosan® 50WP from repeated pulse exposures below prescribed treatment concentrations.
- Preliminary estimates of effects thresholds based upon the predicted exposure profile, the prescribed treatment concentrations and the LC_{50}s after 1 hour exposure indicate that the potential magnitude of effects increases from Interox-Paramove® 50 (active ingredient: hydrogen peroxide) to Salmosan® 50WP (active ingredient: azamethiphos) to AlphaMax® (active ingredient: deltamethrin).
- Laboratory and field estimates of toxicity potential suggest that there is a high risk of lethal effects in exposed non-target organisms that are located immediately adjacent to treated net pens following tarp treatments with AlphaMax® (active ingredient: deltamethrin).

- Given the high number of environmental variables and husbandry factors which can influence the risk of impact, development of field monitoring protocols for exposure and biological effects of therapeutants around aquaculture cages in parallel to laboratory toxicity studies are recommended.

INTRODUCTION

Salmon aquaculture in the Bay of Fundy is situated alongside with other maritime activities, including lobster and herring fisheries. There are currently 90 active marine aquaculture site leases that are regulated by the Province of New Brunswick using a bay management approach to enhance the effectiveness of fish health and environmental management practices.

As in other animal food production systems, it may be necessary to treat aquaculture species for diseases, parasites and fouling organisms. Although management and husbandry practices have evolved over the past 20 years, aquaculture operators still rely on the use of pesticides and drugs (therapeutants) to combat infestations of ecto-parasites, such as sea lice. In the southwest New Brunswick salmon aquaculture industry, and elsewhere in Canada and the world, it is sometimes necessary to control sea lice abundance on cultured Atlantic salmon using therapeutants.

Therapeutant use is regulated in Canada, such that they can only be used under prescription from a licensed veterinarian. The *Food and Drugs Act*, the *Pest Control Products Act*, the *Canadian Environmental Protection Act, 1999*, and the *Fisheries Act* (s. 36) are the three key legislative tools for regulating fish pathogen and pest treatments.

Within the regulatory framework, therapeutants used in the aquaculture industry are classified as either a drug or a pesticide based upon their application method. Products applied topically, or directly into water, are considered pesticides, while products delivered through medicated feed or by injection are considered drugs.

Since 2009, three different anti-sea lice pesticides have been temporarily registered for use in aquaculture in the Bay of Fundy, New Brunswick for various periods of time through the *Pest Control Products Act* Emergency Registration provision. These are: Interox-Paramove® 50[1] (active ingredient (a.i.): hydrogen peroxide); Salmosan® 50WP (a.i.: azamethiphos); and AlphaMax® (a.i.: deltamethrin). In 2011, only the pesticides Interox-Paramove® 50 and Salmosan® 50WP were available for use through Emergency Registrations.

Bath treatments in southwest New Brunswick can be conducted in one of three ways: tarping or skirting salmon net pens, or using well boats. Tarp and skirt bath treatments involve decreasing the volume of water in a salmon net pen by either completely enclosing the net pen in an impervious tarp (tarping), or by surrounding the net pen with an impervious tarp to a depth below that of the salmon (skirting) without enclosing the bottom. The therapeutant is then added to the net pen for the recommended time period, at which point the skirt or tarp is removed and the treated water is released. Well boat bath treatments are conducted by pumping cultured salmon into treatment chambers, or wells, in specially designed boats. The therapeutant is then added to the well for the prescribed time. Following treatment the treated water is discharged from the well into the surrounding water, while the well is simultaneously flushed with fresh seawater. Following flushing, the fish are then pumped back into the net pen.

The therapeutant volume required to treat a single cage is dependent on the application method. For well boat bath treatments, approximately 50% less active ingredient is required relative to tarp treatments. However, treating all fish in a single cage typically requires four well boat treatments, which results in four separate exposure profiles.

[1] Interox-Paramove® 50 is used only in well boats in southwestern New Brunswick.

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A full CSAS advisory process will be undertaken in the future to more broadly define the potential exposure and associated biological effects from aquaculture pest and pathogen treatments.

ANALYSIS

Factors influencing the extent of exposure of non-target organisms to sea lice bath treatments using well boats and tarps.

The exposure of non-target organisms to therapeutants, following sea lice bath treatments, is influenced by the treatment method, as well as a number of physical, chemical, operational and husbandry factors, although some influencing factors are common to both bath treatment methods. In general, dye dispersion studies show that therapeutant dispersion from a treatment site follows an elliptical pattern. The area enclosed within the ellipses associated with tarp releases increases with time in a manner that is consistent with the diffusion diagrams of Okubo (1971, 1974).

In terms of treatment conditions, tarp bath treatments are restricted to periods with relatively weak currents in the tidal cycle (0.1 m/s - <0.5 m/s) and during low wind and wave activity to ensure that tarps do not collapse and trap fish. By comparison, well boat bath treatments can be conducted over a wider range of current and weather conditions.

Influencing Factors

Tarp Bath Treatments

- Therapeutant releases following tarp bath treatments are single point source releases.

The following operational and husbandry factors can influence the exposure profile resulting from therapeutant releases from tarp bath treatments:

- cage size, i.e., diameter, volume
- quantity of therapeutant applied and the therapeutant concentration variability due to mixing (complete or incomplete), i.e., therapeutant concentration at the time of release
- tarp removal, i.e., quickly or slowly removed
- cage net porosity, i.e., mesh size and amount of bio-fouling
- premature termination of the tarp process due to factors such as low oxygen or other concerns related to fish health
- dropping of the pursed net, i.e., are fish retained near surface or allowed to swim throughout full volume of cage
- proximity and layout of other cages and other farm infrastructure

Well Boat Bath Treatments

- Flushing discharges have a finite initial size, a continuous flow for a limited period of time and a decreasing therapeutant concentration over time

The following operational and husbandry factors can influence the exposure profile resulting from therapeutant release from well boat bath treatments:

- well volume
- discharge pipe angle, i.e., horizontal, vertical or at some other angle
- discharge pipe diameter
- discharge pipe depth/height above or below the sea surface
- discharge flow rate, i.e., pumping capacity
- quantity of therapeutant applied, i.e., concentration of source
- discharge solution density - typically the same as the ambient water
- proximity and layout of other cages and farm infrastructure
- degree of bio-fouling on fish cages adjacent to the discharge
- discharge velocity, i.e., the operator can vary this
- discharge direction, i.e., into, or away from cages
- discharge duration, this is under the control of the operator and supervising fish health specialist

Additional observations following well boat and tarp bath treatments:

- The rate of increase in the plume size agreed more closely with predictions when the plume was not influenced by cage infrastructure.
- When cage infrastructure was involved, the plume from tarp bath treatments sometimes underwent a more rapid increase in plume size over the first few minutes to an hour, after which it began to decrease and follow the Okubo rate.

Influencing Factors Common to Both Tarp and Well Boat Bath Treatments

The following physical and chemical factors can influence the exposure profile resulting from therapeutant release from both tarp and well boat bath treatments:

- advective (horizontal) current velocities
- rates of mixing in the horizontal (x,y) and vertical dimensions
    - weather, wind and waves
- proximity of vertical boundaries, including vertical stratification, the sea bottom and inter-tidal zones
- proximity of horizontal boundaries, such as the shoreline, bottom and pycnocline[2]
- chemical behaviour of the therapeutant in the ambient water
- spatial and temporal proximity of multiple bath treatments

What are the known biological effects of hydrogen peroxide (Interox-Paramove® 50), azamethiphos (Salmosan® 50WP) and deltamethrin (AlphaMax®) on key non-target organisms? Known biological effects may include lethal, sub-lethal and behavioural impacts.

Toxicity was evaluated using LC_{50} and $NOEC_{lethal}$ tests based on 1 hour exposures and 95 hours of post-exposure observation for lethal effects. Dilution factors required to reach these thresholds following application of the therapeutant at the recommended treatment concentrations were also included. LC_{50} refers to the concentration where there is 50% mortality of test subjects and $NOEC_{lethal}$ refers to the no observable effect concentration, based on lethality.

[2] Pycnocline: zone where water density has increased rapidly in response to changes in water temperature and salinity, effectively separating surface and deep water.

Interox-Paramove® 50 (active ingredient: hydrogen peroxide (~50%)) is effective against adult and pre-adult sea lice stages, and has a prescribed treatment concentration of up to 1800 mg/L (as active ingredient) for 20–30 minutes depending on water temperatures. The 1 hour LC_{50} ranged from 888 mg/L - 1500 mg/L at ambient water temperatures of 8–12°C for the crustacean species tested (American lobster, Stage I and Adult, Mysid shrimp, and Crangon shrimp), depending on species and life stage (measured concentrations). The 1 hour $NOEC_{lethal}$ values ranged from <187 mg/L for Mysid and Crangon shrimp to 375 mg/L and 2100 mg/L for Stage I and adult American lobster, respectively (measured concentrations).

Salmosan® 50WP (active ingredient: azamethiphos (~47%)) is effective against adult and pre-adult sea lice stages, and is prescribed at 100–150 µg/L (as active ingredient) for 30–60 minutes (depending on temperature). The 1 hour LC_{50} ranged from 30 to >100 µg/L and the $NOEC_{lethal}$ ranged from <0.4–19 µg/L depending on species and life stage, at ambient water temperatures of 8–12°C (measured concentrations).

AlphaMax® (active ingredient: deltamethrin (1%)) is effective against all sea lice stages, and is prescribed at a treatment concentration of 2000 ng/L (as active ingredient) for 30 minutes. The 1 hour LC_{50} ranged from 0.6–27 ng/L and the $NOEC_{lethal}$ ranged from <0.8–5 ng/L at ambient water temperatures (8–12°C), depending on species and life stage (American lobster, Stage I, Stage III and Adult, Mysid shrimp, and Crangon shrimp) (measured concentrations).

In operational conditions, non-target organisms may be exposed to pulsed doses when multiple cages on a farm are treated consecutively. Repeated pulse exposure to Salmosan® 50WP (10 µg/L) at below prescribed treatment concentrations can result in mortality in adult American lobsters and a decreased spawning ability for survivors. Additionally, previous studies have shown that there are seasonal sensitivities due to a combination of factors related to lobster physiology, ambient water temperatures and life stage.

Using the Bay of Fundy experience as a model, what are the predicted biological effects on key non-target species (i.e., how does the exposure profile, including dilution and duration, map to the known biological effects from exposure at that concentration)?

Using the LC_{50} and $NOEC_{lethal}$ biological endpoints derived from 1 hour exposures and 95 hours of post-exposure observation for the species studied, predicted effects at the calculated and/or measured concentrations found in the field studies were analyzed using Risk Quotient (RQ) ratios (exposure concentration: toxicity concentration). RQ ratios are reported as less than, equal to, or greater than 1.0.

These calculations included a number of assumptions, uncertainties and variability, which influence the ratios to varying degrees. This analysis is preliminary with the assumption that exposure is limited to the top 10 m of the water column following a tarp bath treatment, and that calculations are preliminary indicators of the impact associated with single treatments only.

Based on the 1 hour exposures (unless otherwise indicated) and the study parameters, preliminary results for the native crustacean species analyzed suggest:

Interox-Paramove® 50 (active ingredient: hydrogen peroxide)

- Treatment concentrations were weakly toxic to non-target species (i.e., LC_{50} RQ <1.0, except for Mysid shrimp LC_{50} RQ <2.0).
- Well boat flushing will dilute the concentration of Interox-Paramove® 50 to a level where the RQ ratio is <1.0 (i.e., low potential for toxicity).
- Water entrainment associated with flushing discharge jet dynamics is calculated to further dilute Interox-Paramove 50® and further lower the likelihood for toxicity to non-target organisms.

Salmosan® 50WP(active ingredient: azamethiphos)

At a prescribed treatment concentration of 100 µg/L the LC_{50} RQ toxicity potential to non-target species was <1.0 except for adult lobsters (3.1); however, the $NOEC_{lethal}$ RQ ranged from 5.3 (adult lobsters) to > 250 (lobster larvae Stage I).

Post-release, the LC_{50} RQ for adult lobsters was calculated as >1.0 within 100 m of the treatment cage.

Post-release, the $NOEC_{lethal}$ RQ was calculated to remain >1.0 to a distance of 100 m - 1000 m from the treatment cage, depending on species.

For well boat bath treatments, the pumping rate influenced the RQ ratio.

During well boat flushing, the LC_{50} RQ for adult lobsters ranged from <1.0 (at pumping rates ≥100% of capacity) to between 1.0–3.0 at lower pumping rates; for all species tested, the $NOEC_{lethal}$ RQ ranged from >1.0–190 at the end of the pipe (i.e., point of discharge).

Following 20 minutes of flushing, the LC_{50} RQ for adult lobsters was <1.0 for all but the lowest pumping rates (20% of maximum), and the $NOEC_{lethal}$ RQ ranged from 3.0–135 for the two most sensitive species/stages (American lobster Stage I and Mysid shrimp).

Near-field jet plume entrainment via flushing discharge jet dynamics will further dilute the Salmosan® 50WP concentration in the water, and the resulting plume will also result in further dilution (similar to the release plume after tarp treatments).

AlphaMax® (active ingredient: deltamethrin)

- Non-target species mortality was observed following exposure to AlphaMax® (deltamethrin) within and immediately outside cages under operational conditions.
- Predictions based on laboratory toxicity are consistent with this result, with RQ ratios ranging from 14–3333.
- Post-release, the LC_{50} RQ was calculated to remain >1.0 to a distance from the cage edge ranging from 275 m to ≧ 2400 m, depending on species.
- Post-release, the $NOEC_{lethal}$ RQ was calculated to remain >1.0 to a distance from the cage edge of 1000 m to >3000 m.
- From well boats, the AlphaMax® (deltamethrin) concentrations calculated during flushing all exceeded an LC_{50} RQ of 1, and ranged from 4 to >1100. The $NOEC_{lethal}$ RQ ranged from 55–2500 regardless of the pumping rate or species.

At the end of the flushing period, the LC_{50} RQ ratios remained well above 1.0 for all but the highest pumping rate and least sensitive species/stage, while the $NOEC_{lethal}$ RQ ratios were calculated to range from >5 to >1800.

- Near-field jet plume entrainment via flushing discharge jet dynamics will reduce the toxicity potential.

Sources of uncertainty

In order to estimate the exposure profiles from tarp or well boat bath treatments, a number of assumptions were made which are outlined within the research documents, along with their relative importance.

In the Bay of Fundy, presently used well boats have three discharge pipe configurations: including horizontal side discharges, 45 degree angle side discharges, and discharges from the bottom of the vessel bottom. These studies mainly examined horizontal side discharges; hence, as a first approximation, estimates for bottom discharges could be obtained by rotating the side discharge results by 45 and 90 degrees for the other configurations.

There is uncertainty related to the exposure profile for vessels with bottom discharge pipes, but the site bathymetry will be critical in determining the likelihood for benthic interactions. Similarly, therapeutant interactions with the benthic environment following release from tarp treatments are also a source of uncertainty, especially where sites are located close to shore or in shallow water.

Laboratory-based toxicity studies provide an indication of toxicity under carefully controlled parameters. How this translates into the field is uncertain, but some initial studies have been undertaken to try and address this.

CONCLUSIONS AND ADVICE

Exposure from Tarp Treatments

- Releases of treated water from cages may be near instantaneous or spread out over tens of minutes to a few hours, depending on a number of factors, such as the degree of cage biofouling.
- The observed treatment plume dispersion is in general agreement with Okubo predictions after the plume passes through the cage; in the near-field, cage infrastructure can enhance dispersion (Okubo 1971, 1974).
- Transport distances of treated water following bath treatments are highly variable, and depending on site and current conditions, can range from a few tens to thousands (i.e., a few kilometres) of meters, 1–3 hours following release.
- The relative toxicity a therapeutant over time and distance travelled will determine the degree of impact in the near-field and far-field.

Exposure from Well Boat Treatments

- Each well boat discharge is different due to variations in the target treatment concentration, receiving environment, pumping rate and duration, and discharge angle and direction.
- Due to a combination of commercial operating protocols in southwest New Brunswick in which well boats remain at farm sites during discharge, fifty percent (50%) of well boat side-flushing discharges are directed away from the farm infrastructure and 50% are directed into fish pens; as such the exposure profiles from discharges directed into farm infrastructure affects exposure modelling capabilities.
- Study observations are consistent with established jet discharge relationships.

Biological Effects

- American lobster, a commercially important species, was consistently more sensitive to therapeutants than Crangon and Mysid shrimps tested.
- AlphaMax® (deltamethrin) is more toxic to lobsters and other invertebrates than Salmosan® 50WP which is more toxic than Interox-Paramove® 50.
- Sensitivity is species and life stage dependent for all three therapeutants.
- There is evidence that time of year may affect adult lobster sensitivity to Salmosan® 50WP due to a combination of factors related to ambient temperature and species life stage.
- Bi-weekly 1 hour exposures to Salmosan® 50WP at 5 and 10% of treatment concentrations (5 µg/L and 10 µg/L) resulted in significant mortality and reduced spawning success of surviving lobsters. Hence, there is lab-based evidence of sub-lethal

and delayed effects in adult American lobsters from exposure to Salmosan® 50WP concentrations below prescribed treatment concentrations.

Exposure and Effect

- Based on preliminary results, Risk Quotient ratios (exposure concentration: toxicity concentration) were calculated as indicators of the scale of impact associated with single treatments in the Bay of Fundy. Although this approach involves certain assumptions, uncertainties and variability, it has scientific merit.
- Further work is needed to develop a 3-dimensional model to refine the exposure profile, and to predict the potential effects from multiple treatments.
- Overall, the potential magnitude for impact is therapeutant specific: the potential exposure and biological impacts increase from Interox-Paramove® 50 to Salmosan® 50WP to AlphaMax® (deltamethrin).
- Biological impacts on exposed non-target species are dependent upon the species and life stage, environmental conditions and various other influencing factors outlined.

OTHER CONSIDERATIONS

The approach taken in this review was to look at the effects of single releases. Given operation practices can result in multiple treatments occurring over a time scale of hours to days within and among farms, this could lead to the potential for non-target organisms being exposed multiple times.

It will be important for coastal zone management to consider developing indices and levels of acceptable effects for multi-user environments.

An analysis of international approaches, including the scientific foundation of international indices and endpoints, and science advice on their applicability in Canada will provide important additional policy analysis information.

In the longer-term, based on the growing scientific literature, scientifically robust options for practical mitigation practices may need to be assessed.

SOURCES OF INFORMATION

This Science Advisory Report is from the November 2–3, 2011 meeting on *Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments*. Additional publications from this process, including the Proceedings and Research Documents, will be posted as they become available on the DFO Science Advisory Schedule at http://www.dfo-mpo.gc.ca/csas-sccs/index-eng.htm.

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ISSN 1919-5079 (Print)
ISSN 1919-5087 (Online)
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La version française est disponible à l'adresse ci-dessus.

CORRECT CITATION FOR THIS PUBLICATION

DFO. 2013. Guidelines on Defining Potential Exposure and Associated Biological Effects from Aquaculture Pest and Pathogen Treatments: Anti-Sea Lice Bath Treatments in the Bay of Fundy, New Brunswick. DFO. Can. Sci. Advis. Sec. Sci. Advis. Rep. 2012/070.